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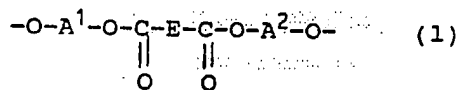
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(54) Title: SYNTHESIS OF OLIGONUCLEOTIDES



(57) Abstract

A method for the synthesis of a plurality of oligonucleotides comprising the steps of (i) forming a first oligonucleotide on a first cleavable link attached to a solid support; (ii) attaching to the first oligonucleotide a cleavable linker moiety; (iii) forming a second oligonucleotide on the cleavable linker moiety; and (iv) cleaving the first cleavable link and the cleavable linker moiety to give a plurality of oligonucleotides; wherein the cleavable linker moiety is of formula (1), in which A¹, A² and E are as defined herein, and novel compounds which may be used in the operation of the method.

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SYNTHESIS OF OLIGONUCLEOTIDES

This invention relates to a method for the synthesis of oligonucleotides and to novel compounds which may be used during operation of the method.

Oligonucleotide sequences are routinely synthesised for use as linkers, adaptors, building blocks for synthetic genes, synthetic regulatory sequences, probes, primers and other purposes and a number of methods have been developed for producing such sequences. These methods rely on the initial attachment of a first suitably protected nucleoside to a solid support by a cleavable linkage followed by sequential reactions of precursors of individual nucleotides to the growing oligonucleotide strand with each addition of a precursor involving a number of chemical reactions. At present the method most generally employed for the production of a lone oligonucleotide is based on phosphoramidite chemistry. This is fully described by Caruthers et al in Tetrahedron Letters 1981, 22, (20) pp 1859-62, by Koster et al in US Patent 4725677 and by M.J. Gait ('Oligonucleotide Synthesis, a Practical Approach', IRL Press Oxford p35-81).

Several types of automated DNA synthesisers are now commercially available which enable an oligonucleotide to be prepared using phosphoramidite chemistry. An illustrative description of how a lone oligonucleotide may be formed by sequential reactions of precursors of the individual nucleotides on a support is provided in the protocol for the Applied Biosystems DNA Synthesiser Model 380B, particularly Section 2 thereof, which is incorporated herein by reference thereto.

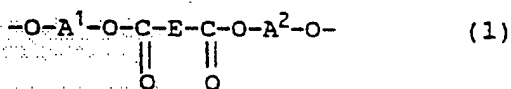
In response to the rapid increase in demand for oligonucleotides, improvements are desirable which will increase the throughput of commercial synthesisers, i.e. increase the number of oligonucleotides synthesised per day.

We have now developed a rapid and efficient method for

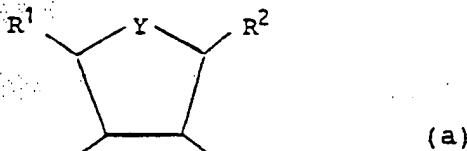
the production of oligonucleotides in which more than one oligonucleotide can be synthesised on the same support using a cleavable linker moiety introduced as required in a growing oligonucleotide chain.

5 According to a first aspect of the present invention there is provided a method for the synthesis of a plurality of oligonucleotides comprising the steps of:

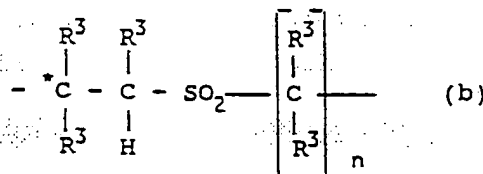
- (i) forming a first oligonucleotide on a first cleavable link attached to a solid support;
- 10 (ii) attaching to the first oligonucleotide a cleavable linker moiety;
- (iii) forming a second oligonucleotide on the cleavable linker moiety; and
- (iv) cleaving the first cleavable link and the cleavable linker moiety to give a plurality of oligonucleotides;
- 15 wherein the cleavable linker moiety is of the Formula (1):



wherein one or both of A^1 and A^2 is a divalent group of the formula (a):



20 R^1 and R^2 are each independently H, optionally substituted alkyl, optionally substituted alkoxy or optionally substituted aryloxy, halogen, cyano, nitro, optionally protected hydroxy, optionally protected oxycarbonyl, optionally protected NH_2 , or an electron withdrawing group; Y is CH_2 , CH_2CH_2 , NH, S or O; E is an organic spacer group; and any remaining group represented by A^1 or A^2 is of the formula (b):



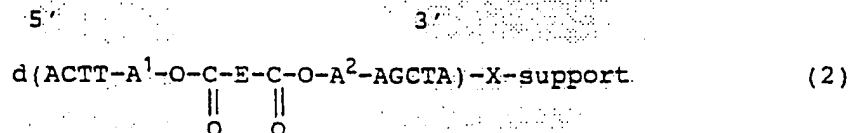
30 wherein n has a value of from 1 to 5; the carbon atom marked with an asterisk is attached to an oxygen atom shown in Formula

(1); and each R³ independently represents H or optionally substituted alkyl.

It is preferred that A¹ and A² are both of Formula (a) and n preferably has a value of from 1 to 3, more preferably 2.

5 The support is preferably a solid support such as is used in automated oligonucleotide synthesis. The preferred solid support is, for example, a modified inorganic polymer such as those disclosed in the US Patent Specification 4,458,066, a silica gel, Porasil C, kieselguhr PDMA, polystyrene, 10 polyacrylamide, Silica CPG (LCAA) or a controlled pore glass as used in, for example, the Applied Biosystems DNA synthesiser Model 380B. The oligonucleotides may be formed from precursors of individual nucleotides by conventional technology used for synthesising oligonucleotides, for example by using 15 phosphoramidite chemistry on an automated oligonucleotide synthesiser as described above. The first oligonucleotide is preferably connected to the support by a hydrolysable group (e.g. a base labile group) as is known in the art.

The first aspect of the invention may be illustrated by 20 the formation of 2 oligonucleotides using different combinations of the phosphoramidites of 2'-deoxyadenosine (dA), 2'-deoxyguanosine (dG), 2'-deoxycytidine (dC), and 2'-deoxythymidine (dT) separated by the cleavable linker moiety of Formula (1) built up sequentially in a 3' to 5' direction from 25 the 3' hydroxy of deoxyribose on a solid support according to the above method described by M.J. Gait. After synthesis the sequence may be represented by:

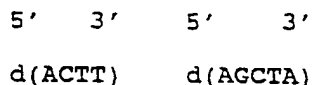


wherein X is a cleavable link.

After cleaving the linker moiety -A¹-O-C-E-C-O-A²- and 30

the link X by which the first oligonucleotide is attached to

the solid support two oligonucleotides result:



Thus two oligonucleotides have been synthesised on a
5 single solid support.

It is preferred that the cleavable linker moiety
connects the first and second oligonucleotides through a 3' and
a 5' oxygen, more preferably through a phosphate, phosphite,
phosphate ester, phosphite ester or H-phosphonate ester, one on
10 each oligonucleotide.

The identity of the first cleavable link is not believed
to be critical, it is preferably base labile, and may be for
example any of the cleavable links used in automated
oligonucleotide synthesisers, such as a link which contains a
15 base labile ester group.

Organic residues of the cleaved linker moiety, such as
hydrocarbon chains, preferably do not remain attached to the
oligonucleotides after cleavage step (iv) to avoid any adverse
affects on the properties of the oligonucleotides which such
20 residues can have.

The first aspect of the invention includes repetition of
steps (ii) and (iii) any desired number of times, for example 1
to 100 times, or preferably 1 to 5 times, to produce further
oligonucleotides which are each connected through a cleavable
25 linker moiety. As will be appreciated, when steps (ii) and
(iii) are repeated the further oligonucleotides are formed on
the cleavable linker moiety attached to the previously formed
oligonucleotide and may be the same as or different to the
previously formed oligonucleotides.

30 The cleavable linker moiety or moieties may be cleaved by
base hydrolysis to give a mixture of individual
oligonucleotides which may, if required, be purified and
separated. Any suitable base may be used, for example, aqueous
ammonia, methylamine.

In this specification the term "oligonucleotide" includes an oligodeoxyribonucleotide, an oligoribonucleotide and analogues thereof (for example those which bear protecting groups), including those with methyl-phosphonate and phosphorothioate or phosphorodithioate diester backbones, and oligonucleotides with oligodeoxyribonucleotides, especially the 2'-oligodeoxyribonucleotides being more usually synthesised by the method of the invention. The preferred oligonucleotides are oligodeoxyribonucleotides, are essentially single stranded, and are preferably from at least two, more preferably at least 5, especially from 10 to 200 bases long.

To users of DNA synthesisers the method of the invention gives the advantage of more effective use of the apparatus and subsequently reducing the cost of production and purification of oligonucleotides.

Thus with the present invention, the DNA synthesiser can produce two or more oligonucleotides (which may be the same or different) on any one of its columns without being re-programmed between each oligonucleotide. Thus when synthesis of one oligonucleotide is completed at a time outside the working day the synthesiser can go on to produce another without any intervention by an operative. This can significantly increase the productivity of such apparatus.

The method of the invention is particularly useful for the synthesis of primers for the Polymerase Chain Reaction (PCR) technique. At present a large proportion of oligonucleotides synthesised are for this purpose. Such primers are typically required in pairs and the method of the invention is convenient since it allows production of oligonucleotides in pairs. This is particularly an advantage when using single column synthesisers and/or for heavily used facilities for out-of hours working.

It is preferred that the precursors of the individual nucleotides are nucleoside phosphoramidites protected at the 5'

oxygen atom and optionally are base protected. Methods of protecting nucleoside bases are known in the art, for example by a protecting group which is removable by treatment with mild acid or alkali. Adenine and cytosine may be protected by an optionally substituted N-benzoyl group and Guanine by an N-isobutyryl group. Thymine and uracil generally do not require protection. Adenine and guanine may also be protected by a dimethylformamide or phenoxyacetyl group, and cytosine by an isobutyryl group. The protecting groups are preferably removed after separation of the protected oligonucleotide from the support. Cleavage of the linker moiety may be effected before, during or after the removal of the protecting groups depending upon the chemistry employed. It is preferred that the protecting groups are removable by treatment with aqueous base, particularly concentrated ammonia solution or methylamine. In a preferred embodiment of the invention the cleavable linker moiety is cleavable under basic or alkaline conditions so that protecting group removal and cleavage of linker moieties can be effected in one step. Typical basic conditions employed, are to mix the protected oligonucleotide with base, for example, concentrated aqueous ammonia, methylamine or a mixture of both, or for example, methanolic sodium hydroxide. The reaction may be carried out, for example at a temperature of room temperature (20°C) to 100°C, more preferably from 50 to 90°C, especially around 55° to 60°C. The reaction is typically carried out over a period of 48 hours, more particularly over a period up to 24 hours, especially from about 5 to 24 hours. It is preferred that a linker moiety is chosen such that cleavage is completed under these conditions.

Other bases, preferably volatile bases may be employed to effect cleavage. These may conveniently be organic amines in water, for example piperidine, diethylamine, or triethylamine, preferably at a concentration from 10-70%.

As examples of precursors of individual nucleotides

suitable for use in the method there may be mentioned the
 2-cyanoethyl-N,N-diisopropylaminophosphoramidites of
 5'-dimethoxytrityl-N-4-benzoyl-2'-deoxycytidine,
 5'-dimethoxytrityl-N-2-isobutyryl-2'-deoxyguanosine,
 5
 5'-dimethoxytrityl-N-6-benzoyl-2'-deoxyadenosine, and
 5'-dimethoxytritylthymidine.

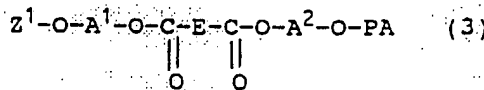
For the synthesis of oligoribonucleotides precursors are,
 for example, the same as for oligodeoxyribonucleotides except
 that on the 2' position of the ribose there is a protected
 10 hydroxyl group, for example a tertiary butyl dimethyl silyloxy
 group or 2'-O-Fpmp phosphoramidites from Cruachem Ltd.

There is an increasing interest in the use of
 oligonucleotides having a 5' phosphate group (see e.g. Higuchi
 & Ockman (1989), Nucl. Acid Res. 17(14), p5865). Therefore a
 15 synthetic method that gives rise to an oligonucleotide having a
 5' phosphate group is of value.

Thus, in a further aspect the method of the present
 invention step (iv) yields desired oligonucleotides each having
 a group selected from hydroxy and phosphate at the 3' and 5'
 20 position.

The cleavable linker moiety of Formula (1) may be
 attached to the first oligonucleotide using a reagent of
 Formula (3) in place of a precursor of an individual nucleotide
 in a manner analogous to conventional oligonucleotide
 25 synthesis.

Accordingly the present invention provides a compound of
 Formula (3):



30 wherein A¹, A² and E are as hereinbefore defined; Z¹ is an acid
 labile protecting group; and -O-PA is a phosphoramidite group,
 a phosphate ester group or a H-phosphonate group.

Suitable acid labile protecting groups represented by Z¹
 will be apparent to those skilled in the art and include those

discussed in 'Protective Groups in Organic Synthesis' by T.W. Greene, Wiley Interscience. Examples of such protecting groups include methoxytrityl (preferably for oligoribonucleotide synthesis only), dimethoxytrityl, pixyl, isobutyloxycarbonyl, t-butyl dimethylsilyl and like protecting groups. Preferably, Z¹ is dimethoxytrityl.

E is preferably an organic spacer group having a length of 2 to 15, more preferably 2 to 6 carbon atoms. E is preferably an alkyl, alkenyl, aryl or aralkyl spacer group, optionally interrupted by an ether, thioether, amino or amido group. Preferred groups represented by E are optionally substituted phenylene, C₂₋₆-alkylene, more preferably -CH₂CH₂-.

R¹ and R² are preferably each independently H; straight or branched chain C₁₋₆-alkyl, especially methyl, ethyl, propyl, butyl or tertiary butyl; or C₁₋₆-alkoxy, especially methoxy, ethoxy, propoxy and butoxy, particularly methoxy; halogen, cyano, nitro, phenoxy, optionally protected hydroxy; optionally protected oxycarbonyl; or optionally protected NH₂.

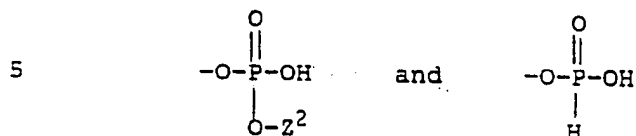
Y is preferably CH₂, CH₂CH₂, NH or O, more preferably CH₂, CH₂CH₂ or O, especially O.

Each R³ independently is preferably C₁₋₆-alkyl, especially methyl, ethyl, propyl or butyl or, more preferably, H. Optional substituents are as defined for R¹ and R².

As examples of groups represented by formula (b) there may be mentioned -*CH₂CH₂-SO₂-CH₂-CH₂- and -*CHCH₃-CH₂-SO₂-CH₂-CH₂-.

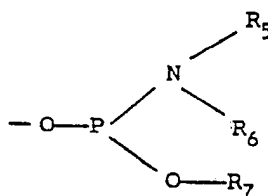
As will be understood, when -O-PA is a H-phosphonate or a phosphoramidite these are oxidised to respectively a phosphate diester or phosphate tri-ester groups during operation of the method, for example using aqueous iodine or peroxide. In the case of H-phosphonate the oxidation is preferably performed after step (iii) and before step (iv), whilst in the case of phosphoramidite it is preferably performed during step (i) and step (iii).

As examples of phosphate ester groups and H-phosphonate groups there may be mentioned groups which, in the free acid form, are respectively of formula:



wherein Z^2 is a protecting group, preferably a base labile protecting group, for example 2-chlorophenyl or 2,4-dichlorophenyl.

10 Preferably, $-\text{O}-\text{PA}$ is a phosphoramidite of general structure:



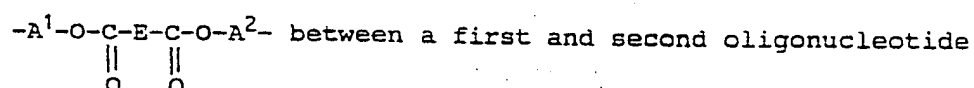
15 wherein R_5 and R_6 are each independently optionally substituted alkyl, especially C_{1-4} -alkyl; optionally substituted aralkyl, especially optionally substituted benzyl; cycloalkyl and cycloalkylalkyl containing up to ten carbon atoms, such as cyclopentyl or cyclohexyl; or R_5 and R_6 taken together with the
20 nitrogen atom to which they are attached form an optionally substituted pyrrolidine or piperidine ring or R_5 and R_6 when taken together with the nitrogen atom to which they are attached form a saturated nitrogen heterocycle which optionally includes one or more additional hetero atom from the group
25 consisting of nitrogen, oxygen and sulphur. R_5 and R_6 are preferably iso-propyl.

R_7 represents a hydrogen atom or a protecting group, for example a phosphate protecting group. As examples of phosphate protecting groups there may be mentioned optionally substituted
30 alkyl groups, for example methyl, 2-cyanoethyl, 2-chlorophenyl, 2,2,2-trihalo-1,1-dimethyl ethyl, 5-chloroquin-8-yl, 2-methylthioethyl and 2-phenylthioethyl groups in which the phenyl ring is optionally substituted, for example by a group selected from halogen, eg. chlorine, or NO_2 . Preferably R_7 is

methyl or, more preferably, 2-cyanoethyl.

As will be appreciated by the skilled person the compounds of the present invention can exist in either the cis or trans form. However, the trans form of the compounds demonstrate a slower rate of cleavage and therefore result in oligonucleotides bearing terminal organic phosphate groups which may find applications in situations where the termini are required to be blocked.

The compounds of Formula (3) are suitable reagents for attaching a cleavable linker moiety of formula



as described by the method of the invention. Under suitable conditions, for example treatment with concentrated ammonium hydroxide, the compounds cleave to give the desired oligonucleotides free from any organic residue of the compound of Formula (3). This is of particular value where oligonucleotides are desired with free or phosphated 3' or 5' termini.

The utility of compounds of Formula (3) can be illustrated by reference to the preparation of the sequence of Formula (2) as discussed above. For example, when A^2 is of Formula (a) the oligonucleotide of formula d(AGCTA) results having a 5'-OH group, and when A^2 is of Formula (b) d(AGCTA) results having a 5'-phosphate group. Accordingly, by appropriate selection of A^2 in a compound of Formula (3) from (a) and (b) the method of the invention provides the great benefit of enabling one to select whether the first, second, and subsequent oligonucleotides prepared according to the method of the invention have a hydroxy group at the 3' position and a hydroxy or phosphate group at the 5' position.

Compounds of Formula (3) wherein -O-PA is a phosphoramidite may be prepared by reacting a compound of the

formula $Z^1-O-A^1-O-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-E-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-O-A^2-OH$ with a compound of formula

X^1-PA in CH_2Cl_2 using di(N-isopropyl)ethylamine as base. PA is preferably a phosphoramidite as defined above for $-O-PA$ except that $-O-$ is absent, and Z^1 , A^1 , E and A^2 are as hereinbefore defined, and X^1 is a leaving group, for example Cl or Br.

When $-O-PA$ in Formula (3) is a phosphate ester group as hereinbefore defined the compound of Formula (3) may be prepared by reaction of a compound of formula

10 $Z^1-O-A^1-O-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-E-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-O-A^2-OH$ with the triazolidine of the

corresponding free phosphate ester using a method analogous to that described in the above book by M.J. Gait.

When $-O-PA$ in Formula (3) is a H-phosphonate group as hereinbefore defined the compound of Formula (3) may be prepared by reaction in a compound of formula

$Z^1-O-A^1-O-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-E-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-O-A^2-OH$ with PCl_3 in the presence of 1,2,4-

triazole using a method analogous to that described by

20 B.C.Froehler et al, Nucleic Acid Research, (1986), 14, 5399-5407.

The compound of formula $Z^1-O-A^1-O-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-E-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-O-A^2-OH$ may be

prepared, for example, by reaction of a compound of formula

25 $Z^1-O-A^1-O-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-E-CO_2H$ with a compound of formula $HO-A^2-OH$,

preferably in an aprotic solvent using a suitable condensing agent such as the aforementioned DCCI or 1-(3-Dimethylamino-propyl)-3-ethylcarbodiimide.

30 The compound of formula $Z^1-O-A^1-O-\overset{\overset{||}{O}}{\underset{\underset{O}{|}}{C}}-E-CO_2H$ may be prepared

by the reaction of the compound of formula Z^1-O-A^1-OH with an activated form of the compound of formula $HO_2C-E-CO_2H$, preferably in an aprotic solvent in the presence of a molar

equivalent of base. The dicarboxylic acid may be activated to attack by the hydroxyl group by being present as the acid anhydride, the acid chloride or some other suitable derivative, or the reaction may be mediated by the presence of a coupling agent as described above.

The compound of formula Z^1-O-A^1-OH may be prepared by the reaction of the compound of formula $HO-A^1-OH$ with Z^1-Cl (or some other suitably activated form of Z^1) in an anhydrous aprotic solvent in the presence of a molar equivalent of base.

When A^1 is of Formula (a) shown above the compound of formula Z^1-O-A^1-OH is preferably prepared by debenzoylation of a compound of formula $Z^1-O-A^1-O-CO-Ph$ in methanol using methylamine. The compound of formula $Z^1-O-A^1-O-CO-Ph$ may be prepared by reaction of compound of formula $HO-A^1-O-CO-Ph$ with Z^1-X^1 , wherein X^1 is a leaving group, for example Cl .

In the above processes for the preparation of the compound of Formula (3), and precursors thereof, Z^1 , A^1 , E , A^2 , O , PA and Z^2 are as hereinbefore defined and DCCI is 1,3-dicyclohexylcarbodiimide.

According to a further aspect of the invention there is provided a compound comprising two or more oligonucleotides linked, preferably by 3' and 5' oxygen atoms, by a group or groups containing a cleavable linker moiety of formula

$-A^1-O-C-E-C-O-A^2-$ wherein A^1 , E and A^2 are as hereinbefore

defined. It is preferred that the cleavable linker moiety of formula $-A^1-O-C-E-C-O-A^2-$ is connected to each

oligonucleotide via a H-phosphonate, phosphate, phosphite, phosphate ester or phosphite ester linkage. It is preferred that one of the oligonucleotides is connected to a support.

H-phosphonate linkages are of formula $-HP(=O)-$, preferred phosphate ester linkages are of formula $-P(=O)-OR_6-$ and preferred phosphite linkages are of formula $-P(-OR_6)-$ wherein R_6

is as hereinbefore defined.

The invention is illustrated by the following non-limiting examples:

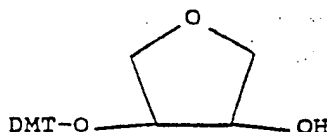
Example 1

5 Preparation of Reagent A1

This was synthesised using the preparation numbered 1 to 4 described below. DMT is 4,4'-dimethoxytrityl.

Step 1 - Preparation of:

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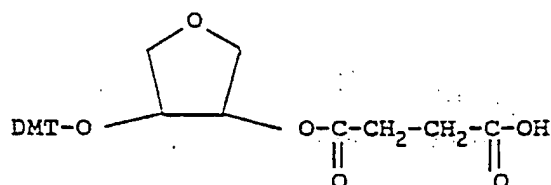
To a solution of 1,4-anhydroerythritol (10.4g, 100mmol) in dry pyridine (100ml) at 0°C, benzoyl chloride (14g, 100mmol) was added dropwise with stirring. When addition was complete, the solution was allowed to warm to room temperature and stirring was maintained for a further two hours. To this solution was added 4,4'-dimethoxytrityl chloride (37.4g, 110mmol) and 4-(N,N-dimethylamino)pyridine (100mg) and the mixture was stirred at room temperature for 16 hours. The solvent was removed by rotary evaporation and the residue redissolved in dichloromethane and washed three times with saturated sodium bicarbonate solution. The dichloromethane solution was dried by the addition of anhydrous sodium sulphate and filtered. The filtrate was evaporated to a gum and redissolved in methanol saturated with methylamine. The resultant solution was incubated at room temperature until no starting material could be detected by TLC. The solvent was removed by rotary evaporation, and the residue redissolved in the minimum volume of dichloromethane/methanol (9/1) and loaded on a silica chromatography column. Elution with the same solvent gave the title compound as a white foam (22g, 54%).

¹H NMR: (δ , CDCl_3): 3.35, 1H, multiplet, CHOH; 3.5, 2H, doublet, $-\text{CH}_2-$; 3.75, 8H, complex multiplet, 2x $-\text{OCH}_3$ and $-\text{CH}_2-$; 4.2, 1H, complex multiplet, DMT-OCH; 6.9, 4H, complex

multiplet, aromatics; 7.25-7.5, 9H, complex multiplet, aromatics.

Step 2 - Preparation of:

5

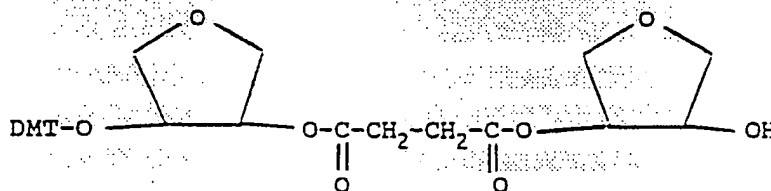


The product from step 1 (10g, 24.6mmol) was dissolved in dry pyridine (150ml) and succinic anhydride (10g, 100mmol) was added. When dissolution was complete, 4-(N,N-dimethylamino)pyridine (500mg) was added and the solution was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and residual pyridine removed by repeated co-evaporation with toluene. The residue was redissolved in dichloromethane (500ml) and washed three times with ice cold 10% citric acid solution. The organic layer was separated, dried (sodium sulphate) filtered and evaporated to a gum which was redissolved in the minimum volume of dichloromethane/methanol (9/1) and loaded on a silica chromatography column. Elution with the same solvent gave the title compound as a white foam (9.7g, 78%).

¹H NMR: (δ, CDCl₃): 2.75, 4H, multiplet, 2x COCH₂; 2.9 and 3.2, 2H, two pseudo triplets, -CH₂-; 3.75, 8H, complex multiplet, 2x -OCH₃ and -CH₂-; 4.2, 1H, complex multiplet, DMT-OCH; 5.0, 1H, multiplet, CHOCO; 6.9, 4H, complex multiplet, aromatics; 7.25-7.5, 9H, complex multiplet, aromatics.

Step 3 - Preparation of:

30

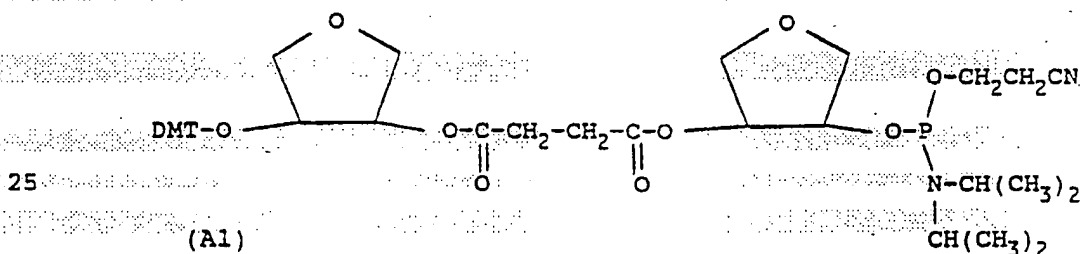


The product from step 2 (10g, 19.5mmol) was dissolved in dry pyridine (200ml) containing 1,4-anhydroerythritol (10.4g, 100mmol). To this solution was added 1-(3-

dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (Aldrich, 3.75g, 19.5mmol). The solution was stirred at room temperature overnight when TLC in dichloromethane:methanol (19:1) showed there to be no starting material present. The solvent was removed under reduced pressure and residual pyridine removed by repeated co-evaporation with toluene. The residue was redissolved in ethyl acetate and washed three times with saturated sodium bicarbonate solution and once with water. The organic layer was separated, dried (sodium sulphate), filtered and evaporated under reduced pressure to give a gum which was redissolved in the minimum volume of dichloromethane:methanol (19:1) and loaded on a silica chromatography column. Elution with the same solvent gave the title compound as a colourless gum (9.5g, 79%).

¹H NMR: (δ, CDCl₃): 2.75-2.9, 5H, multiplet, 2x COCH₂ + -OH; 3.2, 2H, multiplet, -CH₂-; 3.65-3.85, 10H, complex multiplet, 2x -OCH₃ and 2x -CH₂-; 3.9-4.1, 2H, multiplet, -CH₂-; 4.2, 1H, complex multiplet, DMT-O-CH; 4.4, 1H, multiplet, CHOH; 5.0 and 5.1, 2H, two multiplets, 2x CHOCO; 6.9, 4H, complex multiplet, aromatics; 7.25-7.5, 9H, complex multiplet, aromatics.

Step 4 - Preparation of Reagent A1



The product from step 3 (2g, 3.3mmol) was dissolved in dry dichloromethane (50ml) and the solution stirred under a stream of dry argon. To this solution was added dry diisopropylethylamine (2.7ml, 16mmol) and 2-cyanoethyl-N,N-diisopropylaminochlorophosphine (1.05ml, 4.72mmol). The solution was stirred at room temperature under a stream of dry argon for 30 minutes when TLC in dichloromethane:triethylamine (19:1) showed there to be no starting material present. The

reaction was quenched by addition of dry methanol (5ml) and the solution was diluted with ethyl acetate (200ml). This solution was washed with three equal volumes of saturated sodium chloride solution, and one volume of water. The organic layer was separated, dried (sodium sulphate), filtered and evaporated under reduced pressure to a gum which was redissolved in the minimum volume of dichloromethane:triethylamine (19:1) and loaded on a silica chromatography column. Elution with the same solvent gave the title reagent A1 as a colourless gum (1.8g, 65%).

¹H NMR: (δ, CDCl₃): 1.1-1.3, 12H, multiplet, 4x CH₃-; 2.6, 2H, multiplet, CH₂CN; 2.8-3.2, 6H, multiplet, 2x COCH₂ and -CH₂-; 3.5-3.9, 14H, complex multiplet, 2x -OCH₃, 2x CH(CH₃), POCH₂-, 2x -CH₂-; 4.0-4.2, 3H, complex multiplet, -CH₂ and DMT-OCH; 4.5, 1H, multiplet, CH-O-P; 5.0, 1H, multiplet, CHOCO; 5.3, 1H, multiplet, CHOCO; 6.8, 4H, multiplet, aromatics; 7.2-7.5, 9H, complex multiplet, aromatics.

Example 2

Preparation of two oligonucleotides on a single support

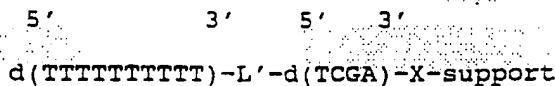
An oligonucleotide was formed on a solid support via a first (conventional) cleavable link using the protocol supplied with the Applied Biosystems 380B DNA synthesiser, using the 3'-(2-cyanoethyl)-N,N-diisopropylaminophosphoramidites of 5'-dimethoxytrityl-N⁴-benzoyl-2'-deoxycytidine, 5'-dimethoxytrityl-N²-isobutyryl-2'-deoxyguanosine, 5'-dimethoxytrityl-N⁶-benzoyl-2'-deoxyadenosine and 5'-dimethoxytritylthymidine (Cruachem) as the precursors of the individual nucleotides. A cleavable linker moiety was attached to the first oligonucleotide by means of reagent A1. Reagent A1 was dissolved in anhydrous acetonitrile to a concentration of 0.1M, and a bottle containing this solution was attached to one of the spare reagent ports on the DNA synthesiser. A column containing controlled pore glass or solid support bearing a 5'-protected nucleoside (in this case deoxyadenosine)

connected by means of a (conventional) cleavable link (succinylglycylglycylaminopropyl, Cruachem) was attached to the synthesiser. The synthesiser was then programmed to synthesise the following sequence:

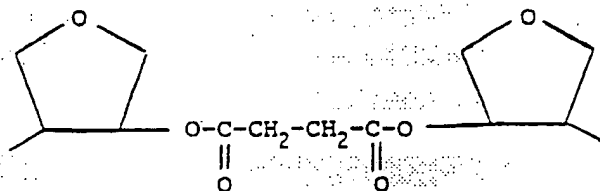
5' (5') TTTTTTTTTT-L'-TCGA (3')

(whereby the cleavable linker moiety L' is introduced by means of reagent A1), using standard synthesis cycles employed on the Applied Biosystems 380B DNA synthesiser. The duration of the reaction steps and the volume of reagents used for coupling, oxidation, capping and detritylation were identical for each coupling, including that of reagent A1. The synthesiser was programmed to perform the conventional concentrated ammonia wash of the column to release the oligonucleotides into collection vials.

15 In this manner the synthesiser achieves the steps of a)
forming a first oligonucleotide of sequence (5'-3') TCGA by
successive reaction of the nucleotide precursors with an
oligonucleotide connected to the controlled pore glass support
via the 3'-OH group and a (conventional) first cleavable link,
20 b) attaching to the first oligonucleotide a cleavable linker
moiety by means of reagent A1, and c) forming a second
oligonucleotide on the cleavable linker moiety having the
sequence (5'-3') TTTTTTTTTT, to give two oligonucleotides
separated by a cleavable linker moiety and bound to a solid
25 support by a cleavable link, as illustrated by the formula:



wherein -L'- is a cleavable linker of formula:



attached to the 5' and 3' oxygen of the first and second oligonucleotides respectively by a group of formula

-P(O)(OCH₂CH₂CN)-O-, and X is a first cleavable link contained in the succinylglycylglycylaminopropyl spacer. The synthesiser also performs the cleavage of the first cleavable link X by the ammonium treatment as in step d) in the method of the invention.

To the vial containing the eluted oligonucleotide in the ammonium solution was added 1ml of 40% aqueous methylamine, and the vial was then incubated at 55°C for 16 hours and evaporated to dryness under reduced pressure. The residue was redissolved in 1ml of water.

Five other oligonucleotides were also synthesised by conventional procedures as described above but omitting the treatment with methylamine. These were designed to represent control molecules to be used in the analysis of products generated by the methylamine treatment above. These oligonucleotides had the following sequences:

- 1) (5') TTTTTTTTTT (3')
- 2) (5') TCGA (3')
- 3) (5') PO₄²⁻ TTTTTTTTTT (3')
- 4) (5') PO₄²⁻ TCGA (3')
- 5) (5') TTTTTTTTTTTTCGA (3')

Thus, oligonucleotides 1) and 2) are of identical length and sequence to the products expected from cleavage of the oligonucleotide containing the cleavable link, and oligonucleotides 3, 4 and 5 are representative of the products expected from partial cleavage of the oligonucleotide containing the cleavable link.

The mixture of oligonucleotides produced in step d) described above was analysed by ion-exchange HPLC on a Pharmacia Mono-Q column using a linear gradient from 0-25% buffer B in buffer A over 35 minutes where buffer A was 50mM Tris-chloride (pH 7.5) and buffer B was 50mM Tris-chloride/800mM sodium chloride (pH 7.5).

Under these conditions, control oligonucleotide 1) had a

retention time of 29 minutes, control sequence 2) had a retention time of 9 minutes, control sequence 3) had a retention time of 32 minutes, control sequence 4) had a retention time of 13 minutes and control sequence 5) had a retention time of 39 minutes.

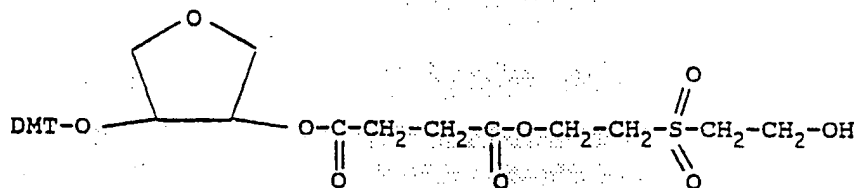
The HPLC profile of the mixture of oligonucleotides produced in step d) above showed only peaks corresponding to the presence of oligonucleotides 1 and 2, thus confirming that complete scission of the cleavable link had occurred, generating the desired products.

Example 3

Preparation of Reagent A2

This was synthesised using the preparations numbered 1 to 2 below. DMT is 4,4'-dimethoxytrityl.

Step 1: Preparation of :

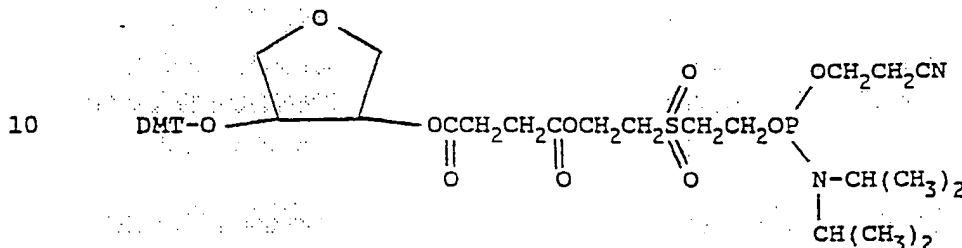


The product from Example 1), step 2) (10g, 19.5 mmol) was dissolved in dry pyridine (200ml) containing 2,2'-sulfonyldiethanol (15g, dried by azeotropic distillation with toluene below 45°C). To this solution was added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (3.75g, 19.5 mmol) and the whole was stirred at room temperature overnight. The solvent was removed under reduced pressure and residual pyridine was removed by repeated co-evaporation with toluene. The gummy residue was redissolved in ethyl acetate (300ml) and washed with saturated sodium bicarbonate (3 x 200ml) and water (200ml). The organic layer was separated, dried (MgSO_4), filtered and evaporated to give a gum which was redissolved in the minimum volume of dichloromethane:methanol (19:1) and applied to a silica column. Elution with the same solvent gave the title compound as a colourless gum (8.2g,

66.3%).

¹H NMR δ (CDCl₃): 2.75 (4H, m, 2 x COCH₂); 2.9-3.3 (4H, m, -CH₂- and -CH₂OH); 3.45 (2H, t, CH₂SO₂); 3.7-3.9 (8H, m, 2 x -OCH₃ and -CH₂-); 4.0 (2H, t, CH₂SO₂); 4.2 (1H, m, DMT-OCH); 4.55 (2H, t, -CH₂OCO); 5.0 (1H, m, CHOCO); 6.9 (4H, m, aromatics); 7.25-7.5 (9H, m, aromatics).

Step 2: Preparation of reagent A2:



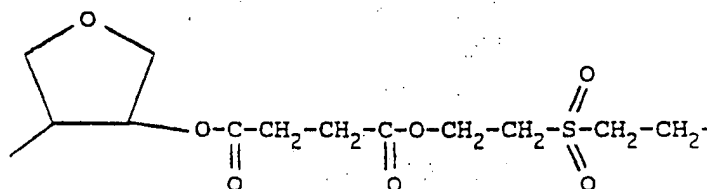
The product from Step 1) (4g, 6.2 mmol) was dissolved in dry dichloromethane (50ml) containing dry diisopropylethylamine (4.4ml, 25 mmol) and the solution was stirred under a stream of dry argon while 2-cyanoethyl-N,N-diisopropylaminochloro-
phosphine (1.67ml, 7.44 mmol) was added dropwise. The solution was stirred under argon at room temperature for a further thirty minutes when TLC in dichloromethane:triethylamine (19:1) showed there to be no starting material present. The reaction was quenched by the addition of dry methanol (5ml), and the solution was diluted with ethyl acetate (200ml). This solution was washed with brine (3 x 200ml) and water (200ml). The organic layer was separated, dried (MgSO₄), filtered and evaporated to a gum which was redissolved in the minimum volume of dichloromethane:hexane:triethylamine (42:55:3) and applied to a silica column. Elution with the same solvent followed by elution with dichloromethane:triethylamine (19:1) gave the title compound as a colourless glass (3.8g, 72.5%).

¹H NMR: δ (CDCl₃): 1.15-1.3 (14H, m, 2 x CH(CH₃)₂); 2.6-2.75 (6H, m, 2 x COCH₂ and CH₂CN); 3.3 (2H, m, -CH₂-); 3.45-3.6 (6H, m, CH₂SO₂ and 2 x CH₂OP); 3.7-4.0 (10H, m, 2 x -OCH₃, -CH₂- and -CH₂SO₂); 4.2 (1H, m, DMT-OCH); 4.5 (2H, m, -CH₂OCO); 5.0 (1H, m, CHOCO); 6.9 (4H, m, aromatics); 7.2-7.5 (9H, m, aromatics).

Example 4)

The method of Example 2) was repeated to synthesise two oligodeoxyribonucleotides bound to a solid support, except that Reagent A2 was used, dissolved in anhydrous acetonitrile to a concentration of 0.1M, in place of Reagent A1.

The two oligonucleotides bound to a solid support by a cleavable link are illustrated by the formula given in Example 2), wherein -L'- is a cleavable linker of formula:

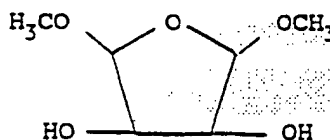


The two oligonucleotides found after step d) in the method of the invention were found to be identical to oligonucleotides 1) and 4) described in Example 2), demonstrating scission of the cleavable link and phosphorylation of the 5'-hydroxyl of the oligonucleotide formed in step a) in the method of the invention.

Example 5)Step 1 - Preparation of Reagent A3

This was synthesised using the preparations numbered a) to f) below. DMT is 4,4'-dimethoxytrityl.

a) Preparation of r-3,Cis-4-dihydroxy-cis plus trans-2,-trans-5-dimethoxytetrahydrofuran



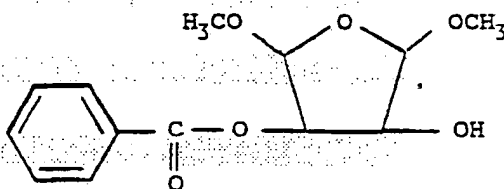
2,5-Dihydro-2,5-dimethoxyfuran (50g; Aldrich, cis/trans mixture) was dissolved in tetrahydrofuran (500ml) in a 5 litre, three-necked flask fitted with a mechanical stirrer. The contents of the flask were cooled to -5°C, and a solution of potassium permanganate (61.9g) in water (2250ml) was added dropwise with vigorous stirring at such a rate as to keep the temperature of the flask contents between 4° and 6°C. This

addition took 80 minutes. The reaction mixture was then left stirring and allowed to warm to room temperature over 15 hours. The precipitated manganese dioxide was filtered off through Celite and washed with THF (200ml). The clear colourless filtrate was evaporated and the residue was shaken vigorously with ethyl acetate (200ml) until no material adhered to the flask wall. The fine precipitate of KOH was filtered off on a glass sinter and washed with ethyl acetate. The filtrate was rotary evaporated to give the title compound as a golden syrup (24.4g, 38.6%). The compound was mainly the meso product (80%) contaminated with the dl compound (20%).

¹³C-NMR:

<u>meso product</u>	<u>CH₃O</u>	55.462ppm
	<u>CHOH</u>	75.303ppm
	<u>CHOCH₃</u>	109.606ppm
<u>dl product</u>	<u>CH₃O</u>	56.451ppm
	<u>CHOH</u>	70.654 and 73.604ppm
	<u>CHOCH₃</u>	103.155 and 108.257ppm

b) Preparation of



The product from step a) above (1.7g) was dissolved in dry pyridine (10ml) and 4-N,N-dimethylaminopyridine (DMAP) (100mg) was added. The mixture was swirled to dissolve the solid and benzoyl chloride (1.2ml) was added. The mixture was then left to stand at 20°C for 16 hours. The mixture was rotary evaporated, and residual pyridine was removed by repeated co-evaporation with toluene. The residual oil was partitioned between ethyl acetate and 1M HCl (40ml of each). The organic layer was washed in succession with water, 1M NaHCO₃, and saturated brine (40ml of each), dried over magnesium sulphate, filtered and rotary evaporated. The

residual oil was redissolved in dichloromethane/methanol (19/1) and applied to a silica column. Elution with the same solvent gave the desired product as a white solid (1.11g, 38%)

¹³C-NMR:

5 CH₃O : 55.680 and 55.951ppm

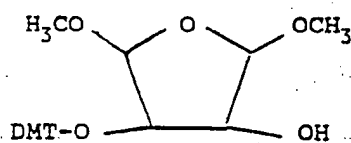
CHOH and CHOCO : 75.016 and 77.973ppm

CHOCH₃ : 107.136 and 109.899ppm

C₆H₅ : 128.576, 129.300, 129.957 and 133.952ppm

CO : 166.076ppm

10 c) Preparation of



The product from step b) above (2.47g), 4,4'-

15 dimethoxytrityl chloride (3.12g) and DMAP (0.15g) were stirred in dry pyridine (20ml) at room temperature for 16 hours. The solvent was removed by rotary evaporation and residual pyridine was removed by repeated co-evaporation with toluene. The residue was partitioned between ethyl acetate and water (50ml
20 of each) and the organic layer was washed with 1M sodium bicarbonate, then saturated brine (20ml of each), dried over magnesium sulphate, filtered and rotary evaporated to an oil which was dissolved in a solution of methylamine in methanol (120ml, 7.5M) and incubated at room temperature for 24 hours.

25 The solution was filtered and the filtrate was evaporated to an oil which was redissolved in the minimum volume of petroleum (b.p. 60-80°C)/ethyl acetate (3/2) and applied to a silica column. Elution with the same solvent gave the desired product (2.3g) as a white foam which was used directly in the next step
30 without further characterisation.

d) Preparation of Reagent A1

The product from step c) above (2.28g) was dissolved in dry pyridine (20ml) and succinic anhydride (0.55g) and DMAP (0.4g) were added. The mixture was swirled to dissolve the

solids and then left at room temperature for 16 hours. The solvent was removed by rotary evaporation and residual pyridine was removed by repeated co-evaporation with toluene. The residue was partitioned between ethyl acetate and 10% (w/v) citric acid (50ml of each). The organic layer was washed with water then saturated brine (50ml of each), dried over magnesium sulphate, filtered and rotary evaporated to an oil which was redissolved in the minimum volume of dichloromethane/methanol (19/1) and applied to a silica column. Elution with the same solvent gave the title compound as a colourless glass (1.98g, 71.4%).

¹³C-NMR:

CH₂ groups of OCCH₂CH₂CO : 28.790ppm and 28.939ppm

CH₃OAr : 54.988ppm

CH₃OCH x 2 : 55.251ppm

CH₃OAr : 56.039ppm,

CHODMT and CHOCO : 75.518ppm and 76.424ppm

quat C of DMT : 106.572ppm and 87.194ppm

CHOCH₃ : 106.572 and 109.102ppm

ArCH : 113.245ppm, 127.821ppm, 128.337ppm, 129.160ppm,

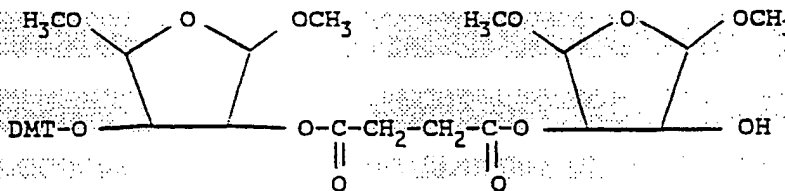
130.250ppm and 130.280ppm

Ar quat C : 139.893ppm, 136.035ppm, 144.855ppm and 158.859ppm

COO : 170.879ppm

COO : 177.540ppm

e) Preparation of :



The product from step d) (1.881g) was dissolved in dry

dichloromethane (5 ml) and N,N'-dicyclohexylcarbodiimide (0.384 g, 0.5 meq) was added. The solid was dissolved by swirling the flask, which was then left at 20°C for one hour.

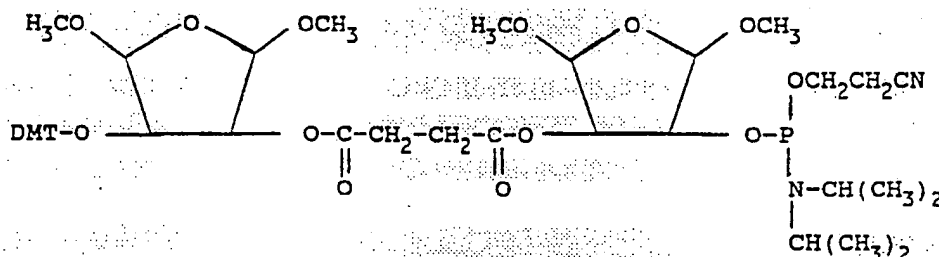
Dicyclohexylurea was filtered off and washed with

dichloromethane (3 x 2 ml). The filtrate and washings were combined and rotary evaporated. To this was added a solution of the product from step 1) (0.708g, 1.27 meq) in dry pyridine (6 ml). When dissolution was complete, the flask was left at 20°C for thirty nine hours, and then rotary evaporated.

Residual pyridine was removed by repeated co-evaporation with toluene. The residual oil was dissolved in the minimum volume of dichloromethane:methanol (96:4), and applied to a silica column. Elution with the same solvent gave the title compound as a yellow foam (0.764g, 63.4%)

$^1\text{H NMR}$ δ (CDCl_3): 2.6-2.8 (4H, m, 2 x COCH_2); 3.25-3.5 (12H, 4s, 4 x $-\text{OCH}_3$); 3.8 (6H, s, 2 x ArOCH_3); 4.0 (1H, DMT- OCH); 4.35 (1H, m, CHOCH_3); 4.45 (1H, m, CHOCH_3); 4.8 (1H, m, CHOH); 5.0 (2H, m, 2 x CHOCO); 5.15 (2H, m, 2 x CHOCH_3); 6.9 (4H, m, aromatics); 7.1-7.55 (9H, m, aromatics).

f) Preparation of Reagent A3:



The product from Step e) (0.5g, 0.702 mmol) was dissolved in dry dichloromethane (15ml) containing dry diisopropylethylamine (0.495 ml, 2.81 mmol) and the solution was stirred under a stream of dry argon while 2-cyanoethyl-N,N-diisopropylaminochlorophosphine (0.189 ml, 0.843 mmol) was added dropwise. The solution was stirred under argon at room temperature for a further thirty minutes when TLC in dichloromethane:triethylamine (19:1) showed there to be no starting material present. The reaction was quenched by the addition of dry methanol (5ml), and the solution was diluted with ethyl acetate (200ml). This solution was washed with brine (3 x 200ml) and water (200ml). The organic layer was separated, dried (MgSO_4), filtered and evaporated to a gum

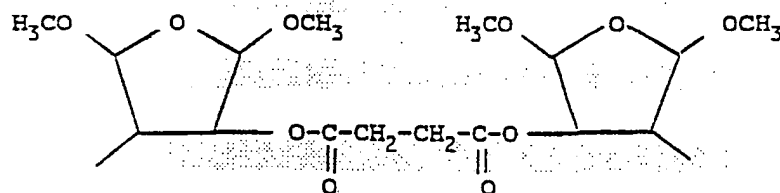
which was redissolved in the minimum volume of dichloromethane:hexane:triethylamine (42:55:3) and applied to a silica column. Elution with the same solvent followed by elution with dichloromethane:triethylamine (19:1) gave the title compound as a colourless glass (0.52g, 81.3%)

$^1\text{H NMR}$ δ (CDCl_3): 1.1-1.3 (14H, m, $2 \times \text{CH}(\text{CH}_3)_2$); 2.5 (2H, m, CH_2CN); 2.6-2.8 (4H, m $2 \times \text{COCH}_2$); 3.2-3.6 (14H, m $4 \times -\text{OCH}_3$ and CH_2OP); 3.8 (6H, s, $2 \times \text{ArOCH}_3$); 4.0 (1H, m, DMT-OCH); 4.3 (1H, m, CHOCH_3); 4.4-4.6 (2H, m, CHOCH_3 and CHOP); 5.0 (2H, m, $2 \times \text{CHOCO}$); 5.2 (2H, m, $2 \times \text{CHOCH}_3$); 6.9 (4H, m, aromatics); 7.1-7.5 (9H, m, aromatics).

Example 6)

The method of Example 2) was repeated to synthesise two oligodeoxyribonucleotides bound to a solid support, except that Reagent A3 was used, dissolved in anhydrous acetonitrile to a concentration of 0.1M, in place of Reagent A1.

The two oligonucleotides bound to a solid support by a cleavable link are illustrated by the formula given in Example 2), wherein $-\text{L}'-$ is a cleavable linker of formula:



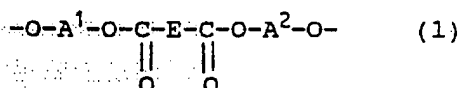
The two oligonucleotides found after step d) in the method of the invention were found to be identical to oligonucleotides 1) and 2) described in Example 2), demonstrating scission of the cleavable link and generation of the desired products.

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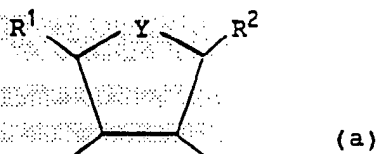
March 23, 1993

CLAIMS

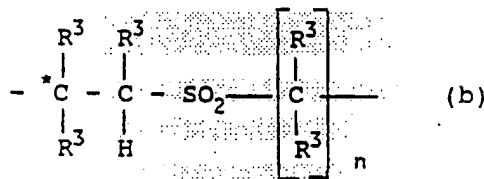
1. A method for the synthesis of a plurality of oligonucleotides comprising the steps of
- (i) forming a first oligonucleotide on a first cleavable link attached to a solid support;
 - (ii) attaching to the first oligonucleotide a cleavable linker moiety;
 - (iii) forming a second oligonucleotide on the cleavable linker moiety; and
 - (iv) cleaving the first cleavable link and the cleavable linker moiety to give a plurality of oligonucleotides; wherein the cleavable linker moiety is of the Formula (1):



wherein one or both of A¹ and A² is a divalent group of the formula (a):

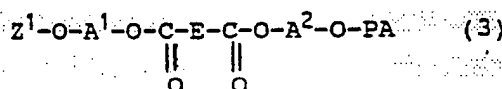


R¹ and R² are each independently H, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted aryloxy, halogen, cyano, nitro, optionally protected hydroxy, optionally protected oxycarbonyl, optionally protected NH₂ or an electron withdrawing group; Y is CH₂, CH₂CH₂, NH, S or O; E is an organic spacer group; and any remaining group represented by A¹ or A² is of the formula (b):

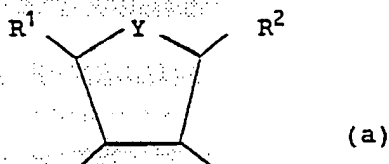


wherein n has a value of from 1 to 5; the carbon atom marked with an asterisk is attached to an oxygen atom shown in Formula (1); and each R³ independently represents H or optionally substituted alkyl.

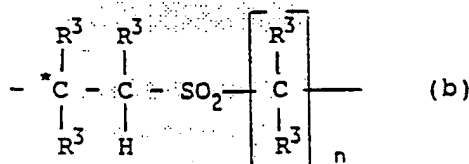
- 2 A method according to claim 1 wherein cleavage of the cleavable linker moiety results in a plurality of oligonucleotides each having at the 3' and 5' position a group selected from hydroxy and phosphate.
- 3 A method according to claim 1 or 2 wherein cleavage of the cleavable linker moiety results in a plurality of oligonucleotides each having a hydroxy at the 3' position.
- 4 A method according to any of claims 1 to 3 wherein steps (i) to (iii) are carried out using an automated oligonucleotide synthesiser and step (iv) is carried out under basic conditions at a temperature from 20 to 100°C for up to 48 hours.
- 5 A method according to any of the preceding claims wherein the cleavable linker moiety of formula (1) is derived from a compound of formula (3) as defined in any of claims 6 to 18.
- 6 A compound of formula (3):



wherein one or both of A¹ and A² is a divalent group of the formula (a):



in which R¹ and R² are each independently H, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted aryloxy, or an electron withdrawing group; Y is CH₂, CH₂CH₂, NH, S or O; E is an organic spacer group; and any remaining group represented by A¹ or A² is of the formula (b):



wherein: n has a value of from 1 to 5; the carbon atom marked with an asterisk is attached to an oxygen atom shown in Formula (3); and each R^3 independently represents H or optionally substituted alkyl; Z^1 is an acid labile protecting group; and -O-PA is a phosphoramidite group, a phosphate ester group or a H-phosphonate group.

7 A compound according to claim 6 wherein A^1 and A^2 are both of formula (a).

8 A compound according to claim 8 wherein A^1 is the group of formula (a) and A^2 is the group of formula (b).

9 A compound according to any of claims 6 to 8 wherein Y is O or CH_2 .

10 A compound according to any of claims 6 to 9 wherein E is an alkyl, aryl or aralkyl spacer group, optionally interrupted by an ether, thioether, amino or amido group.

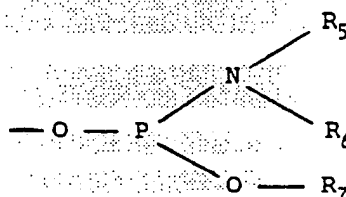
11 A compound according to claim 10 wherein E is optionally substituted phenylene or C_{2-6} -alkylene.

12 A compound according to any of claims 6 to 11 wherein R^1 and R^2 are each independently H, alkyl or alkoxy.

13 A compound according to any of claims 6 to 12 wherein R^3 is independently C_{1-4} -alkyl or H.

14 A compound according to any of claims 6 to 13 wherein Z^1 is dimethoxytrityl.

15 A compound according to any of claims 6 to 14 wherein O-PA is a phosphoramidite of formula

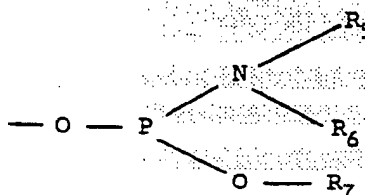


wherein R_5 and R_6 are each independently optionally substituted alkyl, optionally substituted aralkyl, cycloalkyl and cycloalkylalkyl containing up to 10 carbon atoms, or taken together with the nitrogen atom to which

they are attached form an optionally substituted pyrrolidine or piperidine ring and R_5 and R_6 when taken together with the nitrogen atom to which they are attached form a saturated nitrogen heterocycle which optionally includes one or more additional hetero atom(s) from the group consisting of nitrogen, oxygen and sulphur; and R_7 represents a hydrogen or a protecting group.

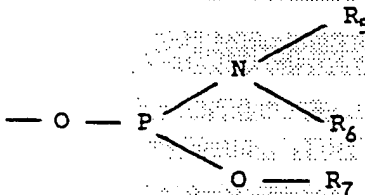
- 16 A compound according to any of claims 6 to 15 wherein the group of formula (a) is where Y is O and R^1 and R^2 are both either hydrogen or methoxy; and the group of formula (b) is $^*CH_2CH_2-SO_2-CH_2-CH_2-$.

- 17 A compound according to any of claims 6 to 16 wherein A^1 and A^2 are both of formula (a), Y is O, R^1 and R^2 are both hydrogen or methoxy, E is $-CH_2CH_2-$, Z^1 is dimethoxytrityl, and O-PA is a phosphoramidite of formula



wherein R_5 and R_6 are each iso-propyl and R_7 is 2-cyanoethyl.

- 18 A compound according to any of claims 6 to 17 wherein A^1 is of formula (a) in which Y is O and R^1 and R^2 are both hydrogen or methoxy and A^2 is $^*CH_2CH_2-SO_2-CH_2-CH_2-$, Z^1 is dimethoxytrityl, and O-PA is a phosphoramidite of formula



wherein R^5 and R^6 are both iso-propyl and R is 2-cyanoethyl.

- 19 A compound comprising two or more oligonucleotides linked by a group or groups containing a cleavable linker moiety

of formula $-A^1-O-C(=O)-E-C(=O)-O-A^2-$ wherein

A^1 , E and A^2 are as defined in any of claims 6 to 18.

20

Use of a compound of formula (3) as defined in any of claims 6 to 18 for synthesis of a plurality of oligonucleotides.

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ <small>According to International Patent Classification (IPC) or to both National Classification and IPC</small> Int.Cl. 5 C07H21/00; C07F9/24											
II. FIELDS SEARCHED <div style="text-align: center; margin-top: 10px;">Minimum Documentation Searched⁷</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 30%; padding: 5px;">Classification System</th> <th style="padding: 5px;">Classification Symbols</th> </tr> <tr> <td style="padding: 5px;">Int.Cl. 5</td> <td style="padding: 5px;">C07H ; C07F</td> </tr> </table> <div style="text-align: center; margin-top: 10px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched⁸</div>			Classification System	Classification Symbols	Int.Cl. 5	C07H ; C07F					
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III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%; padding: 5px;">Category¹⁰</th> <th style="padding: 5px;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 15%; padding: 5px;">Relevant to Claim No.¹³</th> </tr> </thead> <tbody> <tr> <td style="vertical-align: top; padding: 5px;">A</td> <td style="padding: 5px;">EP,A,0 067 597 (ENS BIOLOGICALS INC) 22 December 1982 see page 5 - page 6</td> <td style="vertical-align: top; padding: 5px;">1</td> </tr> <tr> <td style="vertical-align: top; padding: 5px;">P,X</td> <td style="padding: 5px;">WO,A,9 206 103 (IMPERIAL CHEMICAL INDUSTRIES PLC) 16 April 1992 see the whole document</td> <td style="vertical-align: top; padding: 5px;">1-6, 19, 20</td> </tr> </tbody> </table>			Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	A	EP,A,0 067 597 (ENS BIOLOGICALS INC) 22 December 1982 see page 5 - page 6	1	P,X	WO,A,9 206 103 (IMPERIAL CHEMICAL INDUSTRIES PLC) 16 April 1992 see the whole document	1-6, 19, 20
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>											
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search <div style="text-align: center; margin-top: 10px;">27 AUGUST 1993</div> </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; margin-top: 10px;">15. 09. 93</div> </td> </tr> <tr> <td style="padding: 5px;"> International Searching Authority <div style="text-align: center; margin-top: 10px;">EUROPEAN PATENT OFFICE</div> </td> <td style="padding: 5px;"> Signature of Authorized Officer <div style="text-align: center; margin-top: 10px;">DAY G.J.</div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center; margin-top: 10px;">27 AUGUST 1993</div>	Date of Mailing of this International Search Report <div style="text-align: center; margin-top: 10px;">15. 09. 93</div>	International Searching Authority <div style="text-align: center; margin-top: 10px;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center; margin-top: 10px;">DAY G.J.</div>					
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ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

CD 0200604
SA 73927

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0067597	22-12-82	None	
WO-A-9206103	16-04-92	AU-A- 8650991	28-04-92
		CA-A- 2093356	05-04-92
		EP-A- 0552185	28-07-93